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5,10-Methylenetetrahydrofolate Reductase Gene Variants and Congenital Anomalies: A HuGE Review

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The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) is involved in folate metabolism. The MTHFR gene is located on chromosome 1 (1p36.3), and two common alleles, the *C677T* (thermolabile) allele and the *A1298C* allele, have been described. The population frequency of *C677T* homozygosity ranges from 1% or less among Blacks from Africa and the United States to 20% or more among Italians and US Hispanics. *C677T* homozygosity in infants is associated with a moderately increased risk for spina bifida (pooled odds ratio = 1.8; 95% confidence interval: 1.4, 2.2). Maternal *C677T* homozygosity also appears to be a moderate risk factor (pooled odds ratio = 2.0; 95% confidence interval: 1.5, 2.8). The *A1298C* allele combined with the *C677T* allele also could be associated with an increased risk for spina bifida. Some data suggest that the risk for spina bifida associated with *C677T* homozygosity may depend on nutritional status (e.g., blood folate levels, intake of vitamins) or on the genotype of other folate-related genes (e.g., cystathionine-β-synthase and methionine synthase reductase). Studies of the *C677T* allele in relation to oral clefts, Down syndrome, and fetal anticonvulsant syndrome either have yielded conflicting results or have not been yet replicated. *Am J Epidemiol* 2000;151:862–77.

abnormalities; *A1298C*; *C677T*; epidemiology; genetics; 5,10-methylenetetrahydrofolate reductase; neural tube defects; spinal dysraphism

MTHFR GENE AND GENE PRODUCT

The 5,10-methylenetetrahydrofolate reductase (MTHFR) gene is located on chromosome 1 at 1p36.3. The complementary DNA sequence is 2.2 kilobases long and consists of 11 exons (1). Alternative splicing of the gene occurs both in humans and in mice (1). In humans, the major product of the MTHFR gene is a catalytically active 77-kilodalton protein, although a smaller isoform of approximately 70 kilodaltons has been observed in some tissues (2). MTHFR (EC 1.5.1.20) catalyzes the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is the major circulating form of folate (figure 1).

Folic acid and MTHFR are involved in complex biochemical pathways (3) (figure 1). Folate, in its 5-methyl form, participates in single-carbon transfers that occur as part of the synthesis of nucleotides; the synthesis of *S*-adenosyl-methionine; the remethylation of homocysteine to methionine; and the methylation of DNA, proteins, neurotransmitters, and phospholipids. Normal MTHFR activity may help maintain the pool of circulating folate and methionine and possibly prevent a buildup of homocysteine. However, much remains to be learned about folate metabolism. It is also unclear how abnormalities of folate metabolism would cause structural anomalies in the embryo, although both insufficient methylation of crucial metabolites and direct toxicity of homocysteine (4) have been suggested as possible mediators of teratogenesis.

MTHFR GENE VARIANTS

This review focuses on two common MTHFR alleles (*C677T* and *A1298C*) and their association with congenital anomalies. Associations with other conditions such as adult cardiovascular disease, stroke, and coagulation abnormalities are beyond the scope of this review. Also beyond the scope of this review are the other, very rare MTHFR alleles that have been described in patients with homocystinuria, an autosomal recessive metabolic disorder (5–7).

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Abbreviations: CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio.

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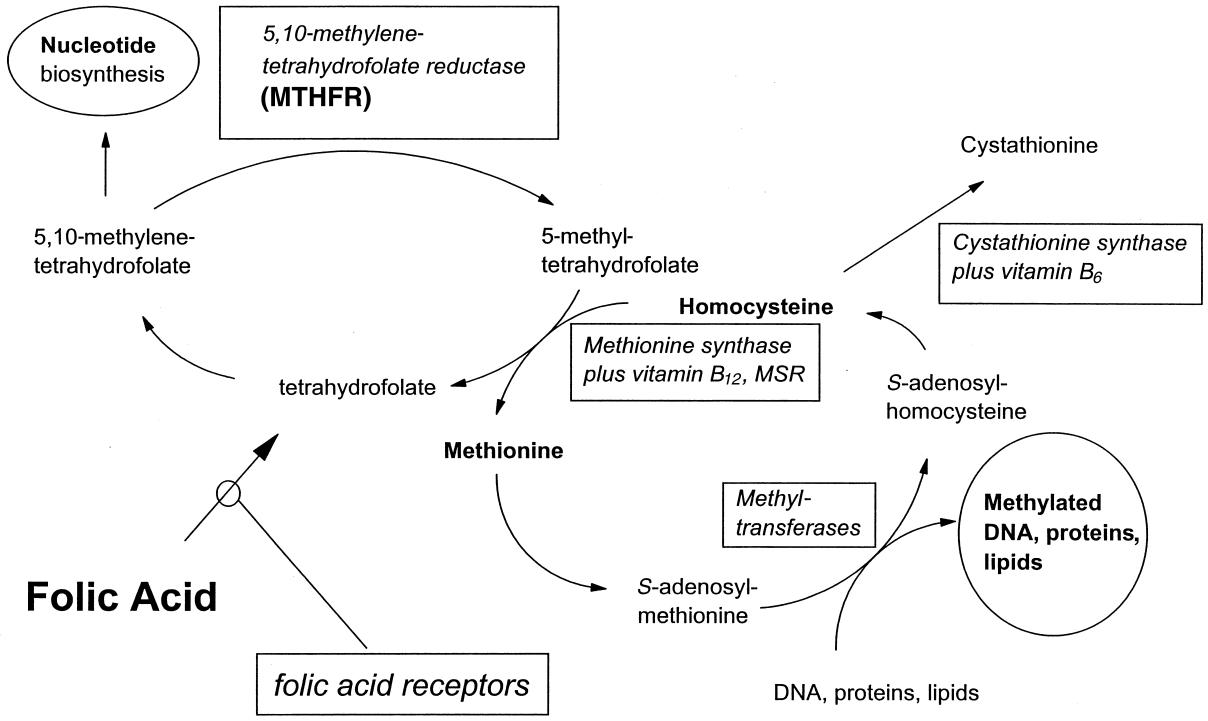

FIGURE 1. Simplified metabolic pathways involving 5,10-methylenetetrahydrofolate reductase (MTHFR). MSR, methionine synthase reductase.

C677T allele

The *C677T* allele is characterized by a point mutation at position 677 of the MTHFR gene that converts a cytosine (C) into a thymine (T); this mutation results in an amino acid substitution (alanine to valine) in the enzyme (2, 8). The *C677T* allele is commonly called "thermolabile," because the activity of the encoded enzyme is reduced at 37°C or more (9). Thus, MTHFR activity among *C677T* homozygotes is 50–60 percent lower at 37°C and approximately 65 percent lower at 46°C than in similarly treated controls (2). Heterozygotes are in the intermediate range. People who are homozygous for the *C677T* allele tend to have mildly increased blood homocysteine levels if their folate intake is insufficient but normal blood levels if their folate intake is adequate (2).

A1298C allele (*C1289A*)

In the *A1298C* allele, a point mutation in exon 7 results in an amino acid substitution (glutamate for alanine) in the enzyme (10–12). This allele has also been called the *C1289A* allele (13). The activity of the encoded enzyme is decreased, although less than is the case with the *C677T* allele (11, 12). People who are homozygous for the *A1298C* allele do not appear to have higher serum homocysteine levels than controls (11, 12). However, people who are compound heterozygous for the *A1298C* and *C677T* alleles (i.e., people with the *A1298C*/*C677T* genotype) tend to have a biochemical profile similar to that seen among *C677T* homozygotes, with increased serum homocysteine levels and decreased serum folate levels (11).

T1059C allele

Very little data are available on the recently described *T1059C* allele (13). The authors of the only published study on this allele remarked that the mutation was silent and that it appeared to be cotransmitted with the *A1298C* mutation (13).

METHODS

Population frequency

For this review, we selected studies that had data on at least 100 individuals, although we included some smaller studies to accumulate otherwise sparse data for particular countries or ethnic groups. From the case-control studies, we selected data only on healthy controls. When a study included potentially heterogenous subsets, such as randomly chosen newborns and healthy adult blood donors (14), we noted which subsets were included in the tables.

Where possible, we recalculated both allele and genotype frequencies from the original data. To compute the confidence intervals of the odds ratios, we used SAS software (Statistical Analysis System, release 6.12; SAS Institute, Cary, North Carolina) that employs Wolff's method to estimate the variance of stratum-specific odds ratios. The allele frequency $F(x)$ of allele x is the proportion of allele x among the $2N$ alleles of the N people in the sample. If these N people include A people who are homozygous for the x allele, B people who are heterozygous, and C people who are neither, then the allele frequency of x can be computed as $F(x) = (2A + B)/(2N)$; the frequency of homozygosity for allele x is A/N, and the frequency of heterozygosity is B/N. We computed the confidence intervals of the allele and genotype frequencies using the normal approximation and the correction for continuity (15). We computed exact confidence intervals when the observed frequency was zero. We also computed pooled estimates of population allele and genotype frequencies by geographic area and race or ethnicity. We excluded some studies from the pooled analysis either because of possible overlap with other studies or because the data were not sufficiently detailed for informative pooling.

Disease associations

To study disease associations, we reviewed the literature for relevant case-control studies, regardless of sample size. Where possible, we recalculated the measures of interest (e.g., odds ratio, attributable fraction) from the original data. For sparse data (e.g., in table 3), we computed Fisher's exact confidence intervals. We used the Mantel-Haenszel method to derive weighted summary odds ratios for disease risk. We excluded some studies from the pooled analysis either because some critical data were missing or because of potential overlap with other studies. Before pooling data from the case-control studies, we used the Breslow-Day test for homogeneity of the odds ratios.

POPULATION FREQUENCIES

C677T allele

Prevalence by geographic region and racial/ethnic group. The population frequency of the *C677T* allele showed regional and ethnic variations (table 1). For example, the allele frequency was high in Italy and among Hispanics living in California and was low among US Blacks and in some areas of sub-Saharan Africa (figure 2). The frequency of *C677T* homozygosity showed similar variability (figure 3). The reason for the high frequency of the allele in many populations is unclear. Table 1 summarizes the data and provides pooled estimates by geographic area. A summary by major ethnic group follows.

Whites. In Europe, the frequency of *C677T* homozygosity ranged from 8 percent in Germany to 18 percent in Italy (16–40) (table 1). In Ireland and Britain, two areas with historically high rates of neural tube defects, the frequencies of homozygosity were 11 percent and 13 percent, respectively. The frequency of homozygosity among Whites outside of Europe (e.g., in Canada, the United States, Brazil, and Australia) ranged from 10 percent to 14 percent (41–53).

Hispanics. The *C677T* allele appears to be very common among Hispanics. Twenty-one percent of Hispanic Whites in a population-based study from California were *C677T*-homozygous (53), as were 25 percent of a convenience sample of Colombians (54).

Blacks. In one study from sub-Saharan Africa that included genotype information, no *C677T* homozygotes were identified among the 234 individuals tested (55). The *C677T* allele frequency was 7 percent (55). In another study of 89 Africans from four tribes in sub-Saharan Africa, the allele frequency was also 7 percent; the frequency of homozygosity was not reported (56). These estimates suggest that the *C677T* allele is less common in African Blacks than in some other ethnic groups. The frequency of *C677T* homozygosity among Blacks living outside of Africa (e.g., in Brazil and the United States) was also low, between 1 and 2 percent (57–62).

Asians. In the pooled analysis of several thousand Japanese (63–71), the frequency of *C677T* homozygosity was 11 percent. Limited data were available for other Asian populations (55).

Amerindians. The *C677T* allele was very common among some but not all of the Amerindian groups studied (55, 56, 72). For example, the frequency of *C677T* homozygosity was 21 percent in a group of Amerindians from Brazil (55), but it was 1 percent in another group, the Tupi Parakana tribe (72).

Other ethnic groups. Data on the distribution of this MTHFR mutation in other populations around the world (73), including some small or isolated ethnic groups (55, 56), are limited. Most of these studies included few people for each population subset, and because it is often unclear how these people were selected, the findings are difficult to interpret.

Prevalence by sex. Most of the studies reviewed did not specify the gender composition of the samples, did not comment on differences in genotype frequencies by sex, or reported that genotype frequency was not significantly different in males and females. One study reported a lower proportion of *C677T* homozygotes among newborn females than among newborn males (74).

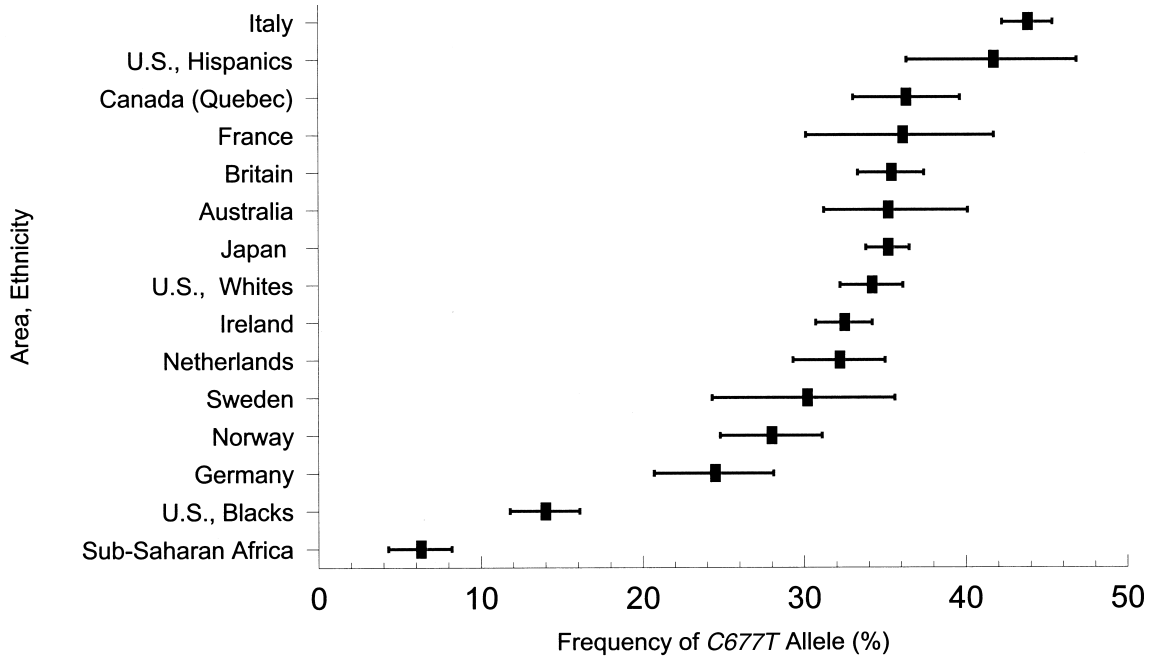

FIGURE 2. Population frequency of the *C677T* allele of 5,10-methylenetetrahydrofolate reductase (MTHFR), by geographic area and ethnicity, 1995–1999. Data were obtained from table 1.

Prevalence by age. One study from Japan reported a lower frequency of the *C677T* allele among older people than among younger people (75). In that study, the frequency of *C677T* homozygosity was 7 percent among people aged 80 years or older, as compared with 14 percent among people aged 55–79 years and 19 percent among people aged 14–55 years (75). In the Netherlands, the frequency of the *C677T* mutation was significantly lower among older men (aged ≥85 years) than among younger men (aged 18–40

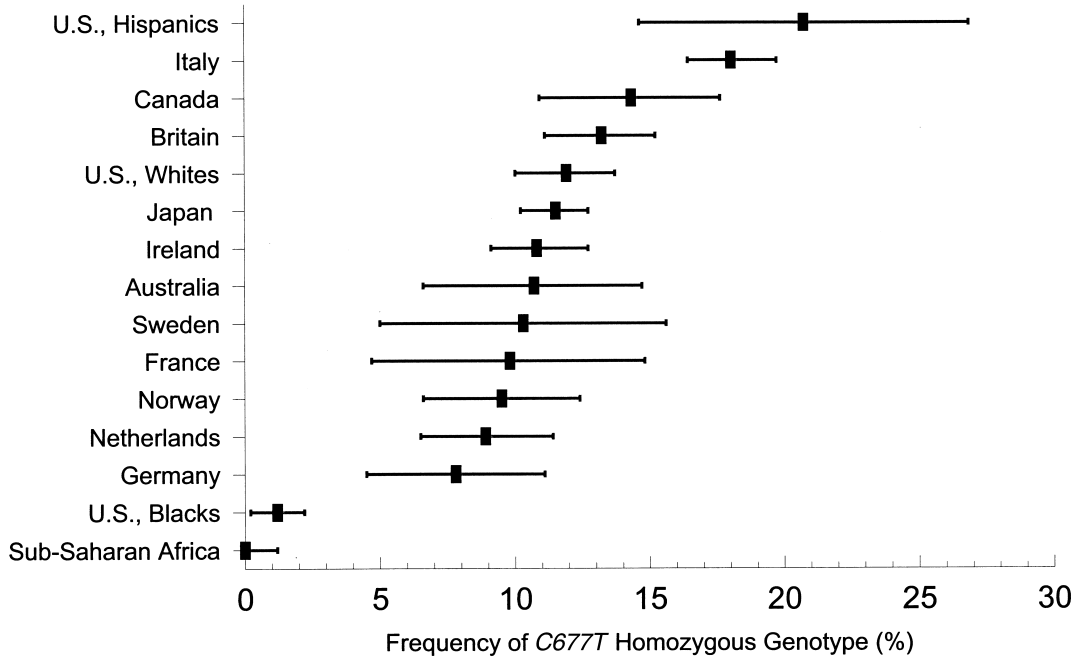

FIGURE 3. Population frequency of homozygosity for the *C677T* allele of 5,10-methylenetetrahydrofolate reductase (MTHFR), by geographic area and ethnicity, 1995–1999. Data were obtained from table 1.

TABLE 1. International distribution of the *C677T* allele of the methylenetetrahydrofolate reductase (MTHFR) gene, by geographic area and ethnicity*

Study area and ethnic group	Total no.	Genotype† (no.)			Allele frequency (%)		Frequency of homozygosity (%)		Year of study	Ref. no.(s)
		TT	CT	CC	Frequency	95% confidence interval	Frequency	95% confidence interval		
Europe										
Britain/Wales										
Britain	199	24	94	81	35.7	30.8, 40.3	12.1	7.5, 16.6	1996	94
Leicester	222	29	97	96	34.9	30.4, 39.2	13.1	8.6, 17.5	1996	17
London	161	22	63	76	33.2	27.9, 38.2	13.7	8.4, 19.0	1997	19
Manchester	164	18	75	71	33.8	28.6, 38.8	11.0	6.2, 15.8	1997	20
Cardiff	300	45	136	119	37.7	33.7, 41.5	15.0	11.0, 19.0	1998	18
Pooled data	1,046	138	465	443	35.4	33.3, 37.4	13.2	11.1, 15.2		
The Netherlands										
The Hague‡	207	10	86	111	25.6	21.3, 29.7	4.8	1.9, 7.8	1995	86
The Hague‡	111	6	42	63	24.3	18.5, 29.7	5.4	1.2, 9.6	1996	22
The Hague	403	38	186	179	32.5	29.2, 35.7	9.4	6.6, 12.3	1998	12
The Hague and other areas‡	1,273	107	537	629	29.5	27.7, 31.2	8.4	6.9, 9.9	1997	108
Rotterdam§	100	7	48	45	31.0	24.3, 37.2	7.0	2.0, 12.0	1997	23
Pooled data	503	45	234	224	32.2	29.3, 35.0	8.9	6.5, 11.4		
France										
France	133	13	70	50	36.1	30.1, 41.7	9.8	4.7, 14.8	1997	95
Germany										
Germany	153	11	40	49	20.3	15.6, 24.6	7.2	3.1, 11.3	1998	91
Jena	104	9	46	49	30.8	24.3, 36.8	8.7	3.3, 14.1	1998	25
Pooled data	257	20	86	151	24.5	20.7, 28.1	7.8	4.5, 11.1		
Ireland and Northern Ireland										
Belfast	625	72	273	280	33.4	30.7, 35.9	11.5	9.0, 14.0	1996	26
Dublin and Belfast	105	7	45	53	28.1	21.8, 33.9	6.7	1.9, 11.4	1996	29
Dublin	318	41	129	148	33.2	29.4, 36.8	12.9	9.2, 16.6	1997	30
Dublin¶	242	20	108	114	30.6	26.4, 34.6	8.3	4.8, 11.7	1997	30
Dublin¶	261	21	121	119	31.2	27.2, 35.1	8.0	4.7, 11.3	1998	92
Pooled data	1,309	141	568	600	32.5	30.7, 34.2	10.8	9.1, 12.5		
Italy										
North (Veneto)‡	137	25	70	42	43.8	37.7, 49.5	18.2	11.8, 24.7	1998	35
North‡	154	35	74	45	46.8	41.0, 52.2	22.7	16.1, 29.3	1997	34
North (Veneto)§	130	17	71	42	40.4	34.2, 46.2	13.1	7.3, 18.9	1997	39
North (Piemonte, Liguria)#	182	27	96	59	41.2	36.0, 46.1	14.8	9.7, 20.0	1998	14
North (Lombardia)	353	74	166	113	44.5	40.7, 48.1	21.0	16.7, 25.2	1997	33
Center (Marche)#	95	17	60	18	49.5	42.1, 56.3	17.9	10.2, 25.6	1998	14
Center (Toscana)	106	32	48	26	52.8	45.9, 59.3	30.2	21.4, 38.9	1998	32
Center (Lazio)	155	25	85	45	43.5	37.9, 48.9	16.1	10.3, 21.9	1998	38
South	150	28	77	45	44.3	38.5, 49.8	18.7	12.4, 24.9	1998	36
South	193	33	102	58	43.5	38.4, 48.3	17.1	11.8, 22.4	1998	16
South	258	39	129	90	40.1	35.8, 44.2	15.1	10.7, 19.5	1996	31
South	431	78	223	130	44.0	40.6, 47.2	18.1	14.5, 21.7	1998	37
Pooled data	2,053	370	1,057	626	43.8	42.2, 45.3	18.0	16.4, 19.7		

Norway										
Norway	323	33	123	167	29.3	25.7, 32.7	10.2	6.9, 13.5	1996	28
Norway	68	4	22	42	22.1	14.7, 28.7	5.9	0.3, 11.5	1997	27
Pooled data	391	37	145	209	28.0	24.8, 31.1	9.5	6.6, 12.4		
Sweden										
Sweden	126	13	50	63	30.2	24.3, 35.6	10.3	5.0, 15.6	1998	40
Africa and the Middle East										
Sub-Saharan Africa										
Sub-Saharan Africa	234	0	31	203	6.6	4.3, 8.8	0.0	0.0, 1.6	1998	55
Sub-Saharan Africa‡	89	NR**	NR	NR	0.07	0.03, 0.11			1998	56
Zaire and Cameroon	67	0	7	60	5.2	1.1, 8.6	0.0	0.0, 5.4	1998	58
Pooled data	301	0	38	263	6.3	4.3, 8.2	0.0	0.0, 1.2		
Middle East										
Yemen	46	1	14	31	17.4	9.1, 24.6	2.2	0.0, 6.4	1998	55
Turkey	93	7	39	47	28.5	21.7, 34.7	7.5	2.2, 12.9	1998	93
Asia										
Asia	346	12	98	236	17.6	14.7, 20.4	3.5	1.5, 5.4	1998	55
Japan	778	79	361	338	33.4	31.0, 35.7	10.2	8.0, 12.3	1997	63, 68
Japan	419	51	214	154	37.7	34.4, 40.9	12.2	9.0, 15.3	1997	64
Japan	325	33	139	153	31.5	27.9, 35.0	10.2	6.9, 13.4	1997	67
Japan	310	42	158	110	39.0	35.1, 42.8	13.5	9.7, 17.4	1998	71
Japan	260	29	135	96	37.1	32.9, 41.2	11.2	7.3, 15.0	1997	66
Japan	146	17	43	86	26.4	21.1, 31.3	11.6	6.4, 16.8	1998	65
Japan	129	14	70	45	38.0	31.9, 43.7	10.9	5.5, 16.2	1996	70
Japan	105	19	51	35	42.4	35.5, 48.8	18.1	10.7, 25.5	1998	69
Japan¶	98	11	49	38	36.2	29.2, 42.7	11.2	5.0, 17.5	1997	66
Pooled data for Japan	2,472	284	1,171		35.2	33.8, 36.5	11.5	10.2, 12.7		
The South Pacific										
Sydney, Australia	225	24	113	88	35.8	31.2, 40.1	10.7	6.6, 14.7	1996	41
The Americas										
Latin America										
Brazil, Whites	107	11	58	38	37.4	30.7, 43.6	10.3	4.5, 16.0	1998	57
Colombians	150	38	70	42	48.7	42.8, 54.2	25.3	18.4, 32.3	1998	54
Brazil, Blacks	137	2	51	84	20.1	15.1, 24.6	1.5	0.0, 3.5	1998	57
Brazil, Blacks	50	1	10	39	12.0	5.1, 17.9	2.0	0.0, 5.9	1998	58
Amerindians (Brazil)	129	10	42	77	24.0	18.6, 29.1	7.8	3.1, 12.4	1998	58
Amerindians (Brazil)	39	8	19	12	44.9	33.2, 55.3	20.5	7.8, 33.2	1998	55
Amerindians (Brazil)	83	1	17	65	11.4	6.3, 16.0	1.2	0.0, 3.6	1998	57
Amerindians (Ecuador)	57	NR	NR	NR	0.43	0.34, 0.52			1998	56
Pooled data for Blacks (Brazil)	187	3	61	123	17.9	13.9, 21.7	1.6	0.0, 3.4		

Table continues

TABLE 1. Continued

Study area and ethnic group	Total no.	Genotype† (no.)			Allele frequency (%)		Frequency of homozygosity (%)		Year of study	Ref. no.(s)
		TT	CT	CC	Frequency	95% confidence interval	Frequency	95% confidence interval		
Canada										
Quebec#	293	46	122	125	36.5	32.5, 40.3	15.7	11.5, 19.9	1996	43
Quebec	121	13	61	47	36.0	29.7, 41.8	10.7	5.2, 16.3	1997	42
Pooled data for Quebec	414	59	183	172	36.3	33.0, 39.6	14.3	10.9, 17.6		
United States										
Whites, California§,#	269	32	122	115	34.6	30.5, 38.5	11.9	8.0, 15.8	1998	53
Whites, Oregon	133	20	63	50	38.7	32.7, 44.4	15.0	9.0, 21.1	1996	43
Whites, South Carolina	151	20	65	66	34.8	29.2, 40.0	13.2	7.8, 18.7	1997	62
Whites, Washington	338	43	141	154	33.6	29.9, 37.1	12.7	9.2, 16.3	1997	52
Whites, unspecified	101	9	43	49	30.2	23.6, 36.3	8.9	3.4, 14.5	1996	61
Whites, unspecified	155	12	78	65	32.9	27.5, 38.0	7.7	3.5, 11.9	1997	45
Pooled data for US Whites	1,147	136	512	499	34.2	32.2, 36.1	11.9	10.0, 13.7		
Blacks, California§,#	17	2	4	11	23.5	7.8, 36.3	11.8	0.0, 27.1	1998	53
Blacks, Georgia	185	4	29	152	10.0	6.8, 12.9	2.2	0.1, 4.3	1998	59
Blacks, Northeast	46	0	9	37	9.8	3.2, 15.3	0.0	0.0, 7.7	1998	60
Blacks, South Carolina	146	0	65	81	22.3	17.3, 26.9	0.0	0.0, 2.5	1997	62
Blacks, unspecified	102	0	20	82	9.8	5.5, 13.6	0.0	0.0, 3.6	1996	61
Pooled data for US Blacks	496	6	127	363	14.0	11.8, 16.1	1.2	0.2, 2.2		
Hispanics (California)§,#	169	35	71	63	41.7	36.3, 46.8	20.7	14.6, 26.8	1998	53
Other races (California)§,#	48	3	16	29	22.9	14.0, 30.8	6.3	0.0, 13.1	1998	53
Boston, Massachusetts, unspecified	188	27	90	71	38.3	33.3, 43.1	14.4	9.3, 19.4	1996	51
South Carolina, unspecified	109	5	36	68	21.1	15.5, 26.3	4.6	0.7, 8.5	1996	89
Physicians, unspecified	290	39	116	135	33.4	29.5, 37.2	13.4	9.5, 17.4	1997	48
Cancer-free subjects	326	49	132	145	35.3	31.5, 38.9	15.0	11.2, 18.9	1997	49
US, unspecified	627	84	263	280	34.4	31.7, 37.0	13.4	10.7, 16.1	1996	46
US, unspecified	170	28	77	65	39.1	33.8, 44.1	16.5	10.9, 22.1	1996	43
US, unspecified	365	45	170	150	35.6	32.1, 39.0	12.3	9.0, 15.7	1996	47
US, unspecified	554	59	238	257	32.1	29.3, 34.8	10.6	8.1, 13.2	1997	44
US, unspecified	500	72	200	228	34.4	31.4, 37.3	14.4	11.3, 17.5	1998	50
Pooled US data, unspecified	3,129	408	1,322	1,399	34.2	33.0, 35.3	13.0	11.9, 14.2		

* For each sample, the table lists the total number of study subjects, their distribution by genotype, and the frequency of the *C677T* allele and of *C677T* homozygosity. Pooled estimates are provided for some groups.

† TT, *C677T* homozygosity; CT, *C677T* heterozygosity; CC, "wild-type" homozygosity.

‡ Not included in the pooled estimate because of insufficient data or possible overlap with other studies listed.

§ Described as population-based.

¶ Pregnant women.

Newborns.

** NR, not reported.

years), though no such difference was found among women (76).

A1298C allele

The population frequency of the *A1298C* allele is less documented than that of the *C677T* allele. A study from Canada (11) and a study from the Netherlands (12) reported similar frequencies of *A1298C* homozygosity—approximately 9 percent—among control subjects. The frequency of *C677T*/*A1298C* compound heterozygosity was 15 percent in the study from Canada (11), 17 percent in a study from the United States (13), and 20 percent in the study from the Netherlands (12).

T1059C allele

The study group that first described the *T1059C* allele (13) reported an allele frequency of 85 percent among control subjects but no genotype frequencies. The researchers also reported that the mutation was silent and that it was apparently cotransmitted with the *A1298C* mutation (13).

DISEASES

Almost all studies on the relation between MTHFR variants and congenital anomalies have focused on neural tube defects. The epidemiology, molecular biology, embryology, and prevention of neural tube defects have been reviewed elsewhere (77, 78). The most common forms of neural tube defects are spina bifida, anencephaly, and encephalocele; other types such as craniorachischisis and iniencephaly occur more rarely. Neural tube defects are thought to result from the failure of the neural tube to close during fetal development. Neural tube defects are severe conditions and can cause early death or severe disability. Spina bifida, for example, is associated with a broad variety of neurologic deficits that disrupt bowel, bladder, sexual, and motor function. The rate of occurrence of neural tube defects is approximately 1 in 1,000 births in many countries, but it varies considerably. The rate is higher in some areas and some ethnic groups (e.g., among Chinese living in northern China and among Hispanics living in some Latin American countries) and lower in others (e.g., among Blacks living in the United States) (78, 79). The lifetime cost of a single case of spina bifida was estimated to be nearly $300,000 US in 1992 (80). Folic acid, if taken daily in a sufficient amount during pregnancy, can reduce the occurrence of neural tube defects by as much as 50–85 percent (81–83).

ASSOCIATIONS

Neural tube defects

The extent to which genetic variation contributes to the occurrence of neural tube defects is unclear. The observance of an association between such occurrences and folic acid use and the report of abnormalities of homocysteine metabolism among some women with a previously affected pregnancy (84, 85) were cues to examine the genetic variation of folate-related genes. To date, most published reports have evaluated the *C677T* allele; fewer have also examined the *A1298C* allele.

C677T allele. In an initial report from the Netherlands (86), homozygosity for the *C677T* allele was associated with an approximately threefold increased risk of being affected with spina bifida or having an affected child (table 2). However, in a later study by the same investigators (12), the association was weaker; the odds ratio for having spina bifida, recalculated from table 1 of the original paper, was 1.7 (95 percent confidence interval (CI): 0.7, 3.9). Subsequent studies from Ireland and the United States (also summarized in table 2) reported a two- to sevenfold increased risk for spina bifida among homozygous children (53, 87–90). Other studies, conducted mostly in Western Europe and the United States, did not find a significant association between homozygosity for the *C677T* allele and risk of neural tube defects (14, 27, 91–95). In these studies, the odds ratios associated with the homozygous genotype among infants or mothers ranged from 0.6 (95 percent CI: 0.2, 2.4) in France (95) to 2.8 (95 percent CI: 0.9, 9.1) in Turkey (93) (table 2). One population-based study also evaluated the association between spina bifida and *C677T* homozygosity among US Hispanics (odds ratio (OR) = 1.9; 95 percent CI: 0.9, 3.9) and Blacks (OR = 1.4; 95 percent CI: 0.04, 3.82) (53), although the sample size for these groups was small (e.g., only eight infants with spina bifida were Black).

For those studies that provided sufficient information, we calculated Mantel-Haenszel pooled estimates of odds ratios (table 2). We excluded one study (86) because of potential overlap with a later, larger study (12). The pooled odds ratio for neural tube defects among infants with the homozygous *C677T* genotype was 1.8 (95 percent CI: 1.4, 2.2). For the heterozygous genotype, the pooled odds ratio was 1.2 (95 percent CI: 0.99, 1.3) (figure 4). The Mantel-Haenszel χ^2 test for trend was significant ($\chi^2 = 13.6$, $p = 0.0002$), suggesting a relation between number of *C677T* alleles and risk of neural tube defects. The pooled attributable fraction associated with *C677T* homozygosity in infants was 7 percent (table 2), though it varied among

TABLE 2. Results of case-control studies of the association between the *C677T* allele of the methylenetetrahydrofolate reductase (MTHFR) gene and risk for spina bifida, by case group (people with a neural tube defect or their parents) and genotype*

Case group and study area	Case genotype†			Control genotype†			TT vs. CC		CT vs. CC		Attributable fraction (%)		Year of study	Ref. no.
	TT	CT	CC	TT	CT	CC	OR‡	95% CI‡	OR	95% CI	TT	CT		
People with neural tube defects														
The Netherlands§	7	26	22	10	86	111	3.53	1.21, 10.3	1.53	0.81, 2.88	9.1	16.2	1995	86
The Netherlands	10	42	34	36	162	205	1.67	0.76, 3.69	1.56	0.95, 2.57	4.7	17.5	1998	12
Ireland	15	32	35	6	43	50	3.57	1.26, 10.1	1.06	0.57, 2.00	13.2	2.2	1995	87
Ireland§	29	NR‡	NR	20	108	114	2.61	1.41, 4.78					1996	88
Ireland§	26	NR	NR	39	NR	NR	2.60	1.50, 4.40					1997	90
Ireland	51	119	101	20	108	114	2.88	1.61, 5.15	1.24	0.86, 1.81	12.3	8.5	1999	107
United Kingdom	5	20	16	24	94	81	1.06	0.35, 3.18	1.08	0.52, 2.22	0.6	3.6	1996	94
France	3	21	19	13	70	50	0.61	0.16, 2.37	0.79	0.39, 1.62			1997	95
Norway	1	15	12	4	22	42	0.88	0.09, 8.58	2.39	0.95, 5.98		31.2	1997	27
Germany	14	44	79	11	40	49	0.79	0.33, 1.88	0.68	0.39, 1.19			1998	91
Turkey	4	25	20	7	39	47	1.34	0.35, 5.10	1.51	0.73, 3.11	2.1	17.2	1998	93
United States	9	15	17	5	36	68	7.20	2.14, 24.3	1.67	0.74, 3.72	18.9	14.7	1996	89
United States¶	41	100	73	72	213	218	1.70	1.07, 2.71	1.40	0.98, 2.00	7.9	13.4	1998	53
Italy	52	89	62	97	313	173	1.50	0.96, 2.33	0.79	0.55, 1.15	8.5		1998	14
Canada	11	26	19	11	44	42	2.21	0.82, 5.99	1.31	0.63, 2.70	10.8	10.7	1999	102
Pooled data							1.75	1.41, 2.18	1.16	0.99, 1.35	7.4	6.0		
Mothers														
The Netherlands§	11	27	32	10	86	111	3.82	1.49, 9.79	1.09	0.61, 1.96	11.6	3.2	1995	86
The Netherlands	18	38	44	36	162	205	2.33	1.21, 4.48	1.09	0.68, 1.77	10.3	3.1	1998	12
United Kingdom	5	15	16	24	94	81	1.06	0.35, 3.18	0.81	0.38, 1.74	0.7		1996	94
United States	9	31	25	6	29	30	1.80	0.56, 5.75	1.28	0.62, 2.67	6.2	10.4	1997	109
Ireland	13	35	34	21	121	118	2.15	0.98, 4.73	1.00	0.59, 1.72	8.5	0.0	1998	92
Ireland	30	108	80	20	108	114	2.14	1.13, 4.03	1.43	0.96, 2.11	7.3	14.9	1999	107
Turkey	7	16	17	7	39	47	2.76	0.85, 9.05	1.13	0.51, 2.53	11.2	4.6	1998	93
Canada	11	27	24	10	36	44	2.02	0.75, 5.43	1.38	0.68, 2.78	9.0	12.0	1999	102
Pooled data							2.04	1.49, 2.81	1.19	0.96, 1.47	8.1	2.0		
Fathers														
The Netherlands§	6	25	29	10	86	111	2.30	0.67, 7.64	1.11	0.58, 2.13	5.7	4.1	1995	86
The Netherlands	8	40	38	36	162	205	1.20	0.52, 2.78	1.33	0.82, 2.17	1.6	11.5	1998	12
United Kingdom	4	14	8	24	94	81	1.69	0.47, 6.09	1.51	0.60, 3.78	6.3	18.2	1996	94
United States	4	27	24	5	24	26	0.87	0.21, 3.61	1.22	0.56, 2.66		8.9	1997	109
Turkey	2	17	14	7	39	47	0.96	0.18, 5.15	1.46	0.64, 3.34		16.2	1998	93
Pooled data							1.18	0.65, 2.12	1.36	0.96, 1.91	1.4	12.3		

* For each study, the table shows the numbers of case and control subjects with each genotype and the risk estimates (odds ratio or attributable fraction).
† TT, *C677T* homozygosity; CT, *C677T* heterozygosity; CC, "wild-type" homozygosity.
‡ OR, odds ratio; CI, confidence interval; NR, not reported.
§ Excluded from the pooled estimate.
¶ Population-based study.

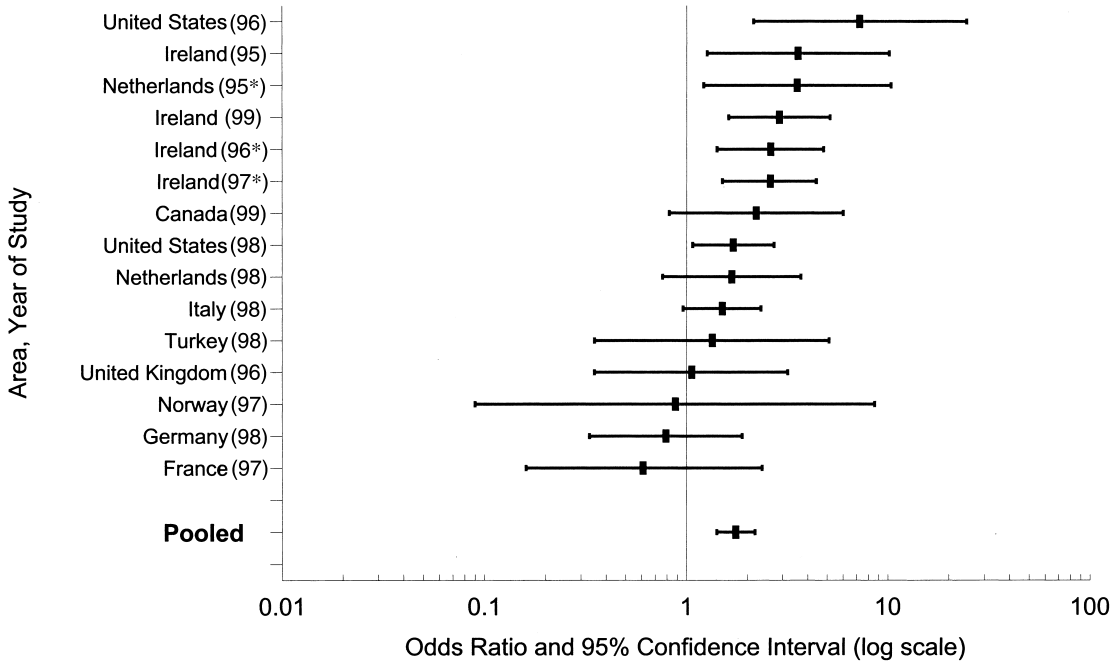

FIGURE 4. Case-control studies that have examined the relation between *C677T* homozygosity and risk of having a neural tube defect, 1995–1999. (*Not included in the pooled estimate.)

studies depending on both the estimated relative risk and the genotype frequency.

The odds ratios for being a mother of an affected child were 2.0 (95 percent CI: 1.5, 2.8) and 1.2 (95 percent CI: 0.96, 1.5) for the homozygous and heterozygous *C677T* genotypes, respectively, and the χ^2 value for trend was statistically significant ($\chi^2 = 7.8$, $p = 0.005$). The father's genotype did not appear to be significantly associated with spina bifida risk (figure 5).

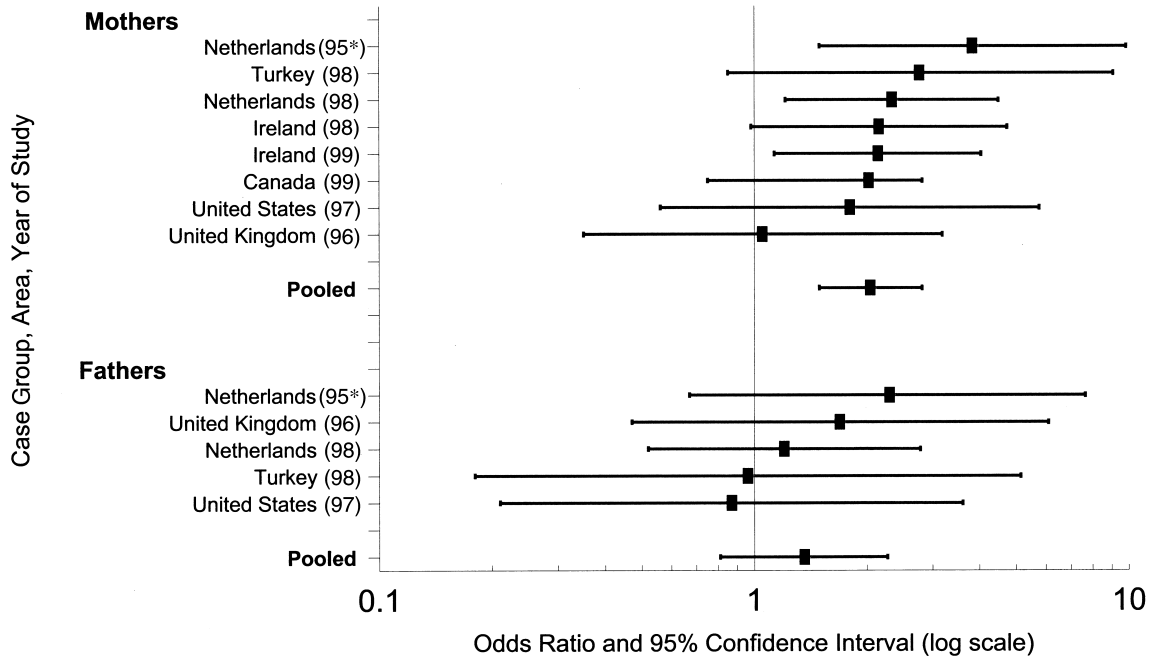

FIGURE 5. Case-control studies that have examined the relation between *C677T* homozygosity and risk of having a child with a neural tube defect, 1995–1999. (*Not included in the pooled estimate.)

A1298C allele. Few studies have evaluated the association between the *A1298C* allele and risk of neural tube defects (12, 13). The findings are not easy to compare, because not all studies have reported complete data. Available data suggest that the *A1298C* allele alone is probably not a major risk factor for spina bifida, with some qualifications (12, 13). A significant association between the *A1298C* allele and spina bifida risk was found in a subset of cases and controls in one study (OR = 2.4; 95 percent CI: 1.4, 4.1); however, this finding could not be replicated in other subsets within the same study (13). Data also suggest that compound heterozygosity for the *C677T* and *A1298C* alleles (i.e., having the *C677T*/*A1298C* haplotype) might be associated with an increased risk for spina bifida in comparison with the presence of two wild-type alleles (12, 13). In one study (12), the odds ratio associated with such compound heterozygosity was 2.0 (95 percent CI: 0.8, 5.1) (recalculated from table 1 of the original report). In another study (13), the corresponding odds ratio appeared to be 2.8 (95 percent CI: 1.1, 7.6) (recalculated from table 13 of the original report).

T1059C allele. In the only known study to date that has examined the *T1059C* allele, this apparently silent polymorphism was associated with an increased risk of neural tube defects (13). However, because this allele was apparently cotransmitted with the *A1298C* allele, the authors cautioned that this association may not be causal. Moreover, the authors noted that the association was found in only one of the three case-control sets in the study and that the finding could not be replicated in the other two sets or in the aggregate data set (13).

Congenital anomalies other than neural tube defects

Few studies have investigated the association between MTHFR genotypes and the risk of congenital anomalies other than neural tube defects (table 3). However, reports of a reduced occurrence of some heart defects, oral clefts, urogenital anomalies, and limb deficiencies among users of supplemental vitamins (96) suggest that such an investigation might yield valuable data.

In a population-based study from California, homozygosity for the *C677T* allele was not associated with an increased risk for cleft lip with or without cleft palate (OR = 0.9; 95 percent CI: 0.6, 1.4) (97). However, in an Irish population, homozygosity for the *C677T* allele was associated with an increased risk for isolated cleft palate (OR = 3.2; 95 percent CI: 1.3, 7.9) and possibly for cleft lip with or without cleft palate (OR = 1.6; 95 percent CI: 0.8, 3.4) (98).

TABLE 3. Results of case-control studies of the association between the *C677T* allele of the methylenetetrahydrofolate reductase (MTHFR) gene and risk for congenital anomalies other than neural tube defects, by case group (people with an anomaly or their parents) and genotype*

Anomaly and case group	Study area and ethnic group	Genotype of cases†			Genotype of controls†			TT vs. CC		CT vs. CC		Attributable fraction (%)		Year of study	Ref. no.
		TT	CT	CC	TT	CT	CC	OR‡	95% CI‡	OR	95% CI	TT	CT		
Cleft lip, with or without cleft palate															
Affected persons§	United States, Whites	25	73	93	25	116	84	0.90	0.46, 1.17	0.57	0.37, 0.88			1998	97
Affected persons§	United States, Hispanics	12	45	31	21	47	35	0.65	0.25, 1.65	1.1	0.55, 2.14	1.1		1998	97
Affected persons§	United States, Blacks	1	1	6	2	3	8	0.67	0.01, 16.2	0.44	0.01, 7.63			1998	97
Affected persons	Ireland	10	NR‡	NR	83	NR	NR		1.65 (0.81, 3.35)¶			6.0		1999	98
Cleft palate															
Affected persons	Ireland	7	NR	NR	83	NR	NR		3.23 (1.32, 7.86)¶			17.9		1999	98
Down syndrome															
Mothers	United States and Canada	8	34	15	4	22	24	3.20	0.69, 16.79	2.47	0.99, 6.24	9.6	35.5	1999	99
Fetal anticonvulsant syndrome															
Affected persons	Scotland	3	28	15	14	65	73	1.04	0.17, 4.44	2.10	0.98, 4.60	0.6	20.2	1999	100
Mothers	Scotland	9	16	11	14	65	73	4.27	1.28, 13.79	1.63	0.66, 4.19	19.1	17.2	1999	100
Fathers	Scotland	2	11	6	14	65	73	1.74	0.16, 11.03	0.20	0.02, 1.00	4.5		1999	100

* For each study, the table shows the numbers of case and control subjects with each genotype and the risk estimates (odds ratio or attributable fraction).
† TT, *C677T* homozygosity; CT, *C677T* heterozygosity; CC, "wild-type" homozygosity.
‡ OR, odds ratio; CI, confidence interval; NR, not reported.
§ Population-based study.
¶ Odds ratio comparing *C677T* homozygosity with heterozygosity. Numbers in parentheses, 95% confidence interval.

One study from the United States and Canada reported an increased prevalence of *C677T* heterozygosity and homozygosity among 57 mothers of infants with Down's syndrome in comparison with 50 control mothers (99). The odds ratios for having a child with Down syndrome were 3.2 (95 percent CI: 0.7, 16.8) for *C677T* homozygotes and 2.5 (95 percent CI: 1.0, 16.8) for *C677T* heterozygotes, respectively. The interpretation of these findings is limited by the use of convenience samples of case and control subjects. Moreover, the frequency of *C677T* homozygosity among mothers of cases (14 percent) was not substantially higher than that observed in population studies in the United States and Canada, whereas the frequency among control mothers (8 percent) appeared to be lower than would be expected based on their ethnic distribution.

A study from Scotland assessed the prevalence of the *C677T* allele in mothers who had children with fetal anticonvulsant syndrome (100). Such mothers were more likely to be homozygous for the allele than were a group of adult controls (25 percent vs. 9 percent), though this was not the case for the affected children and the children's fathers (table 3).

INTERACTIONS

Because of the genetic complexity of folate metabolism, MTHFR alleles may be expected to interact with other folate-related genes and with folate consumption (figure 1).

Gene-environment interactions

Several observations have suggested that the risk of neural tube defects associated with MTHFR genotypes may vary depending on nutritional status. For example, in one study (53), maternal vitamin use was associated with a reduced risk for spina bifida among both infants with normal alleles (OR = 0.30) and those with the homozygous *C677T* genotype (OR = 0.20). Those findings are consistent with the presence of an interaction between genotype and vitamin use (53, 101), because *C677T* homozygosity was associated with a markedly increased risk of spina bifida when the mother had taken no multivitamin supplements during the periconceptional period (OR = 5.2; 95 percent CI: 2.2, 12.6) but not when the mother had used multivitamins (OR = 1.2; 95 percent CI: 0.3, 4.3). Another study (102) further suggested the possibility of gene-nutrient interactions by reporting that the combination of *C677T* homozygosity with a red blood cell folate level in the lowest quartile was associated with a 13-fold increased risk for spina bifida (OR = 13.4; 95 percent CI: 2.5, 72.3).

Gene-gene interactions

Genes whose alleles have been studied in conjunction with MTHFR alleles include the genes for cystathione-β-synthase (90), methionine synthase (13, 102), and methionine synthase reductase (103). Located at 21q22.2, the cystathione-β-synthase gene encodes an enzyme that catalyzes the conversion of homocysteine to cystathionine, thus providing an outflow route for homocysteine (figure 1). In one study from Ireland that evaluated the *844Ins68* allele of the cystathione-β-synthase gene in conjunction with the *C677T* allele of the MTHFR gene (90), the authors concluded that the cystathione-β-synthase allele did not contribute to spina bifida risk (90). However, other researchers noted that in that study, the presence of mutations in both genes was associated with an increased risk for spina bifida (OR = 5.2; 95 percent CI: 1.4, 21.2) that appeared to be higher than expected based on the risk associated with the cystathione-β-synthase variant alone (OR = 0.8; 95 percent CI: 0.4, 1.4) or the MTHFR variant alone (OR = 2.1; 95 percent CI: 1.1, 3.9); this suggested the presence of a gene-gene interaction (104). Similar findings were reported by other investigators (105). In a study of the *Gly919* allele of methionine synthase and the *C677T* allele of MTHFR, mothers who had both gene variants were at increased risk for having a child with spina bifida (OR = 3.9; 95 percent CI: 1.0, 16.3) (106). In another study that evaluated the *A66G* allele of methionine synthase reductase and the *C677T* allele of MTHFR, children who were homozygous for both gene variants also appeared to be at increased risk for spina bifida (OR = 4.1; 95 percent CI: 1.0, 16.4) (103).

Maternal-fetal interactions

The finding that *C677T* homozygosity in the mother but not in the father is associated with an increased risk for spina bifida in the infant (table 2) suggests that the mother's genotype might independently contribute to her child's risk of disease. To examine this possibility, researchers have studied how the risk for spina bifida varies depending on the genotype of both mother and child. So far, however, the studies are few, the data limited, and the findings contradictory. For example, the results of one study (102) suggested that such maternal-fetal interactions may occur. In that study, the risk of spina bifida was increased sixfold (OR = 6.0; 95 percent CI: 1.3, 28.5) when both mother and infant had the homozygous *C677T* genotype, as compared with the case when both mother and infant were homozygous for the "wild-type" allele; when the mother was homozygous for the *C677T* allele but not the infant, the odds ratio was 1.3

(95 percent CI: 0.27, 9.62). However, another study (107) showed no such effect.

LABORATORY TESTS

The common MTHFR alleles can be identified by direct sequencing. However, because these alleles create (*C677T*) or abolish (*A1298C*) specific restriction sites, they are more commonly identified by first digesting the polymerase chain reaction-amplified genomic DNA with the appropriate restriction enzyme (*Hinf*I for the *C677T* allele (8) and *Mbo*II for the *A1298C* allele (10)) and then separating the fragments using gel electrophoresis. These procedures have been performed on DNA extracted from many sources, including blood, dried blood spots, and amniotic fluid. We have been unable to find data on the specificity, sensitivity, and predictive value of these tests for classifying the underlying genotype.

POPULATION TESTING

The usefulness of clinical or population testing for MTHFR variants has been neither evaluated nor advocated. Before such testing can be considered, the disease risk associated with MTHFR alleles and their interaction with other genes and environmental factors must become better known.

VALIDITY AND BIAS

Of the many studies of MTHFR alleles, few have been population-based (13, 53). Most studies have relied on convenience samples of subjects and have often provided little information on how such samples were selected (91, 93–95). When sources are described, they typically include selected hospitals (27) and birth defects research centers or parent organizations (12, 14, 86–90, 108). Researchers have also enrolled people from prospective cohorts of pregnant women or infants within defined geographic areas (92). These factors caution against considering the current results conclusive, and they underscore the potential for bias. For example, biased results could originate if population stratification were to occur in a case-control study—i.e., if case and control subjects were selected from populations with different underlying allele frequencies. A few studies have acknowledged this potential problem and have used a family-based approach and the transmission disequilibrium test to assess the role of different alleles in spina bifida risk (13, 94, 106, 109). Finally, the presence of an association, regardless of whether it was detected using a population- or family-based approach, does not necessarily imply a causal relation.

GAPS AND RESEARCH PRIORITIES

Many basic questions on the role of MTHFR alleles in health and disease remain unanswered. Research priorities should include the following:

Provide valid and precise estimates of MTHFR genotype frequencies. Large and representative samples, such as can be assembled by randomly selecting blood spots from a population-based metabolic screening program, can generate valid and precise estimates of genotype frequencies among well-defined populations. To allow for comparisons and meta-analyses, researchers should report the actual distribution of the genotypes that were studied, not only percentages or allele frequencies.

Assess absolute, relative, and attributable risks of disease. Relative risks, absolute risks, and attributable fractions need to be determined for each health outcome and genotype of interest. Carefully conducted population-based studies can provide such risk estimates.

Search for additional genes and alleles. Only a small fraction of spina bifida cases are attributable to known MTHFR alleles. Additional genetic and environmental risk factors for folate-preventable neural tube defects remain to be identified.

Evaluate more outcomes. The basic metabolic role of folate and homocysteine suggests that genetic variation in folate-related genes may also play a role in the etiology of congenital anomalies other than neural tube defects. Population-based case-control studies of congenital anomalies in which biologic samples were collected would provide an efficient mechanism for evaluating this hypothesis.

Explore interactions. Finding the causes of congenital anomalies and other multifactoral conditions will probably require a careful study of gene-gene and gene-environment interactions. The study of gene-nutrient interactions in particular may provide critical data for prevention. In addition to standard design issues, researchers planning to study interactions should give special consideration to sample size requirements.

CONCLUDING REMARKS

The etiology of most common birth defects has long been described as multifactorial, yet the multiple factors involved have been difficult to find. The study of MTHFR may help define some of such factors. The findings related to MTHFR also underscore the potential usefulness of studying common gene variants in relation to common exposures, and highlight the need for collaborative efforts between epidemiologists, clinicians, and laboratory scientists.

REFERENCES

1. Goyette P, Pai A, Milos R, et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome 1998;9:652–6.
2. Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb Haemost 1997;78:523–6.
3. Rosenblatt DS. Inherited disorders of folate transport and metabolism. In: Scriver CR, Beaudet AL, Sly WS, et al, eds. The metabolic and molecular bases of inherited disease. New York, NY: McGraw-Hill Book Company, 1995:3111–28.
4. Rosenquist TH, Ratashak SA, Selhub J. Homocysteine induces congenital defects of the heart and neural tube: effect of folic acid. Proc Natl Acad Sci U S A 1996;93:15227–32.
5. Goyette P, Sumner JS, Milos R, et al. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat Genet 1994;7:195–200.
6. Goyette P, Frosst P, Rosenblatt DS, et al. Seven novel mutations in the methylenetetrahydrofolate reductase gene and genotype/phenotype correlations in severe methylenetetrahydrofolate reductase deficiency. Am J Hum Genet 1995;56:1052–9.
7. Goyette P, Christensen B, Rosenblatt DS, et al. Severe and mild mutations in cis for the methylenetetrahydrofolate reductase (MTHFR) gene and description of five novel mutations in MTHFR. Am J Hum Genet 1996;59:1268–75.
8. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. (Letter). Nat Genet 1995;10:111–13.
9. Kang SS, Wong PW, Susmano A, et al. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet 1991;48:536–45.
10. Viel A, Dall'Agnese L, Simone F, et al. Loss of heterozygosity at the 5,10-methylenetetrahydrofolate reductase locus in human ovarian carcinomas. Br J Cancer 1997;75:1105–10.
11. Weisberg I, Tran P, Christensen B, et al. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998;64:169–72.
12. van der Put NM, Gabreels F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 1998;62:1044–51.
13. Trembath D, Sherbondy AL, Vandyke DC, et al. Analysis of select folate pathway genes, PAX3, and human T in a Midwestern neural tube defect population. Teratology 1999;59:331–41.
14. de Franchis R, Buoninconti A, Mandato C, et al. The *C677T* mutation of the 5,10-methylenetetrahydrofolate reductase gene is a moderate risk factor for spina bifida in Italy. J Med Genet 1998;35:1009–13.
15. Fleiss JL. Statistical methods for rates and proportions. New York, NY: John Wiley and Sons, Inc, 1981.
16. Ames PR, Tommasino C, D'Andrea G, et al. Thrombophilic genotypes in subjects with idiopathic antiphospholipid antibodies—prevalence and significance. Thromb Haemost 1998;79:46–9.
17. Adams M, Smith PD, Martin D, et al. Genetic analysis of thermolabile methylenetetrahydrofolate reductase as a risk factor for myocardial infarction. QJM 1996;89:437–44.
18. Bowen DJ, Bowley S, John M, et al. Factor V Leiden (*G1691A*), the prothrombin 3′-untranslated region variant (*G20210A*) and thermolabile methylenetetrahydrofolate reductase (*C677T*): a single genetic test genotypes all three loci—determination of frequencies in the S. Wales population of the UK. Thromb Haemost 1998;79:949–54.
19. Markus HS, Ali N, Swaminathan R, et al. A common polymorphism in the methylenetetrahydrofolate reductase gene, homocysteine, and ischemic cerebrovascular disease. Stroke 1997;28:1739–43.
20. Salden A, Keeney S, Hay CR, et al. The *C677T* MTHFR variant and the risk of venous thrombosis. (Letter). Br J Haematol 1997;99:472.
21. Kluijtmans LA, Kastelein JJ, Lindemans J, et al. Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. Circulation 1997;96:2573–7.
22. Kluijtmans LA, van den Heuvel LP, Boers GH, et al. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. Am J Hum Genet 1996;58:35–41.
23. Verhoef P, Kok FJ, Kluijtmans LA, et al. The 677C→T mutation in the methylenetetrahydrofolate reductase gene: associations with plasma total homocysteine levels and risk of coronary atherosclerotic disease. Atherosclerosis 1997;132:105–13.
24. Brulhart MC, Dussoix P, Ruiz J, et al. The (Ala-Val) mutation of methylenetetrahydrofolate reductase as a genetic risk factor for vascular disease in non-insulin-dependent diabetic patients. (Letter). Am J Hum Genet 1997;60:228–9.
25. Reinhardt D, Sigusch HH, Vogt SF, et al. Absence of association between a common mutation in the methylenetetrahydrofolate reductase gene and the risk of coronary artery disease. Eur J Clin Invest 1998;28:20–3.
26. Harmon DL, Woodside JV, Yarnell JW, et al. The common "thermolabile" variant of methylene tetrahydrofolate reductase is a major determinant of mild hyperhomocysteinaemia. QJM 1996;89:571–7.
27. Bjorke-Monsen AL, Ueland PM, Schneede J, et al. Elevated plasma total homocysteine and *C677T* mutation of the methylenetetrahydrofolate reductase gene in patients with spina bifida. QJM 1997;90:593–6.
28. Guttormsen AB, Ueland PM, Nesthus I, et al. Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia (≥40 μmol/liter): The Hordaland Homocysteine Study. J Clin Invest 1996;98:2174–83.
29. Gallagher PM, Meleady R, Shields DC, et al. Homocysteine and risk of premature coronary heart disease: evidence for a common gene mutation. Circulation 1996;94:2154–8.
30. Molloy AM, Daly S, Mills JL, et al. Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red-cell folates: implications for folate intake recommendations. Lancet 1997;349:1591–3.
31. de Franchis R, Mancini FP, D'Angelo A, et al. Elevated total plasma homocysteine and 677C→T mutation of the 5,10-methylenetetrahydrofolate reductase gene in thrombotic vascular disease. (Letter). Am J Hum Genet 1996;59:262–4.
32. Abbate R, Sardi I, Pepe G, et al. The high prevalence of thermolabile 5-10 methylenetetrahydrofolate reductase (MTHFR) in Italians is not associated to an increased risk for coronary artery disease (CAD). Thromb Haemost 1998;79:727–30.
33. Sacchi E, Tagliabue L, Duca F, et al. High frequency of the *C677T* mutation in the methylenetetrahydrofolate reductase (MTHFR) gene in Northern Italy. (Letter). Thromb Haemost 1997;78:963–4.
34. Cattaneo M, Tsai MY, Bucciarelli P, et al. A common mutation in the methylenetetrahydrofolate reductase gene (*C677T*) increases the risk for deep-vein thrombosis in patients with mutant factor V (factor V:Q506). Arterioscler Thromb Vasc Biol 1997;17:1662–6.
35. Girelli D, Friso S, Trabetti E, et al. Methylenetetrahydrofolate reductase *C677T* mutation, plasma homocysteine, and folate in subjects from northern Italy with or without angiographically documented severe coronary atherosclerotic disease: evidence for an important genetic-environmental interaction. Blood 1998;91:4158–63.
36. Grandone E, Margaglione M, Colaizzo D, et al. Presence of FV Leiden and MTHFR mutation in a patient with complicated pregnancies. (Letter). Thromb Haemost 1997;77:1036–7.
37. Margaglione M, D'Andrea G, d'Addedda M, et al. The meth-

ylenetetrahydrofolate reductase *TT677* genotype is associated with venous thrombosis independently of the coexistence of the FV Leiden and the prothrombin *A20210* mutation. Thromb Haemost 1998;79:907–11.
38. Motti C, Gnasso A, Bernardini S, et al. Common mutation in methylenetetrahydrofolate reductase: correlation with homocysteine and other risk factors for vascular disease. Atherosclerosis 1998;139:377–83.
39. Tosetto A, Missiaglia E, Frezzato M, et al. The VITA project: *C677T* mutation in the methylene-tetrahydrofolate reductase gene and risk of venous thromboembolism. Br J Haematol 1997;97:804–6.
40. Kostulas K, Crisby M, Huang WX, et al. A methylenetetrahydrofolate reductase gene polymorphism in ischaemic stroke and in carotid artery stenosis. Eur J Clin Invest 1998;28:285–9.
41. Wilcken DE, Wang XL, Sim AS, et al. Distribution in healthy and coronary populations of the methylenetetrahydrofolate reductase (MTHFR) *C677T* mutation. Arterioscler Thromb Vasc Biol 1996;16:878–82.
42. Christensen B, Frosst P, Lussier-Cacan S, et al. Correlation of a common mutation in the methylenetetrahydrofolate reductase gene with plasma homocysteine in patients with premature coronary artery disease. Arterioscler Thromb Vasc Biol 1997;17:569–73.
43. Deloughery TG, Evans A, Sadeghi A, et al. Common mutation in methylenetetrahydrofolate reductase: correlation with homocysteine metabolism and late-onset vascular disease. Circulation 1996;94:3074–8.
44. Anderson JL, King GJ, Thomson MJ, et al. A mutation in the methylenetetrahydrofolate reductase gene is not associated with increased risk for coronary artery disease or myocardial infarction. J Am Coll Cardiol 1997;30:1206–11.
45. Brugada R, Marian AJ. A common mutation in methylenetetrahydrofolate reductase gene is not a major risk of coronary artery disease or myocardial infarction. Atherosclerosis 1997;128:107–12.
46. Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res 1996;56:4862–4.
47. Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996;93:7–9.
48. Ma J, Stampfer MJ, Hennekens CH, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. Circulation 1996;94:2410–16.
49. Ma J, Stampfer MJ, Giovannucci E, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57:1098–102.
50. Verhoef P, Rimm EB, Hunter DJ, et al. A common mutation in the methylenetetrahydrofolate reductase gene and risk of coronary heart disease: results among U.S. men. J Am Coll Cardiol 1998;32:353–9.
51. Schmitz C, Lindpaintner K, Verhoef P, et al. Genetic polymorphism of methylenetetrahydrofolate reductase and myocardial infarction: a case-control study. Circulation 1996;94:1812–14.
52. Schwartz SM, Siscovick DS, Malinow MR, et al. Myocardial infarction in young women in relation to plasma total homocysteine, folate, and a common variant in the methylenetetrahydrofolate reductase gene. Circulation 1997;96:412–17.
53. Shaw GM, Rozen R, Finnell RH, et al. Maternal vitamin use, genetic variation of infant methylenetetrahydrofolate reductase, and risk for spina bifida. Am J Epidemiol 1998;148: 30–7.
54. Camacho Vanegas O, Giusti B, Restrepo Fernandez CM, et al. Frequency of factor V (FV) Leiden and *C677T* methylenetetrahydrofolate reductase (MTHFR) mutations in Colombians. (Letter). Thromb Haemost 1998;79:883–4.
55. Schneider JA, Rees DC, Liu YT, et al. Worldwide distribution of a common methylenetetrahydrofolate reductase mutation. (Letter). Am J Hum Genet 1998;62:1258–60.
56. Pepe G, Camacho Vanegas O, Giusti B, et al. Heterogeneity in world distribution of the thermolabile *C677T* mutation in 5,10-methylenetetrahydrofolate reductase. (Letter). Am J Hum Genet 1998;63:917–20.
57. Arruda VR, Siqueira LH, Goncalves MS, et al. Prevalence of the mutation C677→T in the methylene tetrahydrofolate reductase gene among distinct ethnic groups in Brazil. Am J Med Genet 1998;78:332–5.
58. Franco RF, Araujo AG, Guerreiro JF, et al. Analysis of the 677 C→T mutation of the methylenetetrahydrofolate reductase gene in different ethnic groups. Thromb Haemost 1998; 79:119–21.
59. Dilley A, Austin H, Hooper WC, et al. Relation of three genetic traits to venous thrombosis in an African-American population. Am J Epidemiol 1998;147:30–5.
60. Giles WH, Kittner SJ, Ou CY, et al. Thermolabile methylenetetrahydrofolate reductase polymorphism (*C677T*) and total homocysteine concentration among African-American and white women. Ethn Dis 1998;8:149–57.
61. McAndrew PE, Brandt JT, Pearl DK, et al. The incidence of the gene for thermolabile methylene tetrahydrofolate reductase in African Americans. Thromb Res 1996;83:195–8.
62. Stevenson RE, Schwartz CE, Du YZ, et al. Differences in methylenetetrahydrofolate reductase genotype frequencies between Whites and Blacks. (Letter). Am J Hum Genet 1997; 60:229–30.
63. Morita H, Taguchi J, Kurihara H, et al. Gene polymorphism of 5,10-methylenetetrahydrofolate reductase as a coronary risk factor. J Cardiol 1997;29:309–15.
64. Arinami T, Yamada N, Yamakawa-Kobayashi K, et al. Methylenetetrahydrofolate reductase variant and schizophrenia/depression. Am J Med Genet 1997;74:526–8.
65. Neugebauer S, Baba T, Watanabe T. Methylenetetrahydrofolate reductase gene polymorphism as a risk factor for diabetic nephropathy in NIDDM patients. (Letter). Lancet 1998;352:454.
66. Sohda S, Arinami T, Hamada H, et al. Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. J Med Genet 1997;34:525–6.
67. Morita H, Kurihara H, Tsubaki S, et al. Methylenetetrahydrofolate reductase gene polymorphism and ischemic stroke in Japanese. Arterioscler Thromb Vasc Biol 1998; 18:1465–9.
68. Morita H, Taguchi J, Kurihara H, et al. Genetic polymorphism of 5,10-methylenetetrahydrofolate reductase (MTHFR) as a risk factor for coronary artery disease. Circulation 1997;95:2032–6.
69. Nakata Y, Katsuya T, Takami S, et al. Methylenetetrahydrofolate reductase gene polymorphism: relation to blood pressure and cerebrovascular disease. Am J Hypertens 1998; 11:1019–23.
70. Nishio H, Lee MJ, Fujii M, et al. A common mutation in methylenetetrahydrofolate reductase gene among the Japanese population. Jpn J Hum Genet 1996;41:247–51.
71. Ou T, Yamakawa-Kobayashi K, Arinami T, et al. Methylenetetrahydrofolate reductase and apolipoprotein E polymorphisms are independent risk factors for coronary heart disease in Japanese: a case-control study. Atherosclerosis 1998;137:23–8.
72. Arruda VR, von Zuben PM, Chiaparini LC, et al. The mutation Ala677→Val in the methylene tetrahydrofolate reductase gene: a risk factor for arterial disease and venous thrombosis. Thromb Haemost 1997;77:818–21.
73. Gudnason V, Stansbie D, Scott J, et al. *C677T* (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): its frequency and impact on plasma homocysteine concentration in different European populations. EARS Group. Atherosclerosis 1998;136:347–54.
74. Rozen R, Fraser FC, Shaw G. Decreased proportion of female newborn infants homozygous for the 677 C→T muta-

tion in methylenetetrahydrofolate reductase. (Letter). Am J Med Genet 1999;83:142–3.
75. Matsushita S, Muramatsu T, Arai H, et al. The frequency of the methylenetetrahydrofolate reductase-gene mutation varies with age in the normal population. (Letter). Am J Hum Genet 1997;61:1459–60.
76. Heijmans BT, Gussekloo J, Kluft C, et al. Mortality risk in men is associated with a common mutation in the methylenetetrahydrofolate reductase gene (MTHFR). Eur J Hum Genet 1999;7:197–204.
77. Sells CJ, Hall JG. Neural tube defects. Ment Retard Dev Disabil Res Rev 1998;4:239–307.
78. Botto LD, Moore CA, Khoury MJ, et al. Neural tube defects. N Engl J Med 1999;341:1509–19.
79. International Clearinghouse for Birth Defects Monitoring Systems. Annual report 1998. Rome, Italy: International Centre for Birth Defects, 1998.
80. Waitzman NJ, Scheffler RM, Romano PS. The cost of birth defects: estimates of the value of prevention. Lanham, MD: University Press of America, 1996.
81. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 1992;327:1832–5.
82. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet 1991;338:131–7.
83. Berry RJ, Li Z, Erickson JD, et al. Preventing neural tube defects with folic acid in the People's Republic of China. N Engl J Med 1999;341:1485–90.
84. Mills JL, McPartlin JM, Kirke PN, et al. Homocysteine metabolism in pregnancies complicated by neural-tube defects. Lancet 1995;345:149–51.
85. Steegers-Theunissen RP, Boers GH, Trijbels FJ, et al. Maternal hyperhomocysteinemia: a risk factor for neural-tube defects? Metabolism 1994;43:1475–80.
86. van der Put NM, Steegers-Theunissen RP, Frosst P, et al. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet 1995;346:1070–1.
87. Whitehead AS, Gallagher P, Mills JL, et al. A genetic defect in 5,10-methylenetetrahydrofolate reductase in neural tube defects. QJM 1995;88:763–6.
88. Kirke PN, Mills JL, Whitehead AS, et al. Methylenetetrahydrofolate reductase mutation and neural tube defects. (Letter). Lancet 1996;348:1037–8.
89. Ou CY, Stevenson RE, Brown VK, et al. 5,10-Methylenetetrahydrofolate reductase genetic polymorphism as a risk factor for neural tube defects. Am J Med Genet 1996;63:610–14.
90. Ramsbottom D, Scott JM, Molloy A, et al. Are common mutations of cystathionine beta-synthase involved in the aetiology of neural tube defects? Clin Genet 1997;51:39–42.
91. Koch MC, Stegmann K, Ziegler A, et al. Evaluation of the MTHFR *C677T* allele and the MTHFR gene locus in a German spina bifida population. Eur J Pediatr 1998;157:487–92.
92. Molloy AM, Mills JL, Kirke PN, et al. Low blood folates in NTD pregnancies are only partly explained by thermolabile 5,10-methylenetetrahydrofolate reductase: low folate status alone may be the critical factor. Am J Med Genet 1998;78: 155–9.
93. Boduroglu K, Alikasifoglu M, Anar B, et al. 677→CT mutation on the methylenetetrahydrofolate reductase gene is not a risk factor for neural tube defects in Turkey. (Letter). Arch Dis Child Fetal Neonatal Ed 1998;78:F235.
94. Papapetrou C, Lynch SA, Burn J, et al. Methylenetetrahydrofolate reductase and neural tube defects. (Letter). Lancet 1996;348:58.
95. Mornet E, Muller F, Lenvoise-Furet A, et al. Screening of the *C677T* mutation on the methylenetetrahydrofolate reductase gene in French patients with neural tube defects. Hum Genet 1997;100:512–14.
96. Hall J, Solehdin F. Folic acid for the prevention of congenital anomalies. Eur J Pediatr 1998;157:445–50.
97. Shaw GM, Rozen R, Finnell RH, et al. Infant *C677T* mutation in MTHFR, maternal periconceptional vitamin use, and cleft lip. Am J Med Genet 1998;80:196–8.
98. Mills JL, Kirke PN, Molloy AM, et al. Methylenetetrahydrofolate reductase thermolabile variant and oral clefts. Am J Med Genet 1999;86:71–4.
99. James SJ, Pogribna M, Pogribny IP, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr 1999;70:495–501.
100. Dean JC, Moore SJ, Osborne A, et al. Fetal anticonvulsant syndrome and mutation in the maternal MTHFR gene. Clin Genet 1999;56:216–20.
101. Botto LD, Mulinare J. Re: "Maternal vitamin use, genetic variation of infant methylenetetrahydrofolate reductase, and risk for spina bifida." (Letter). Am J Epidemiol 1999;150: 323–4.
102. Christensen B, Arbour L, Tran P, et al. Genetic polymorphisms in methylenetetrahydrofolate reductase and methionine synthase, folate levels in red blood cells, and risk of neural tube defects. Am J Med Genet 1999;84:151–7.
103. Wilson A, Platt R, Wu Q, et al. A common variant in methionine synthase reductase combined with low cobalamin (vitamin B12) increases risk for spina bifida. Mol Genet Metab 1999;67:317–23.
104. Botto LD, Mastroiacovo P. Exploring gene-gene interactions in the etiology of neural tube defects. Clin Genet 1998;53: 456–9.
105. de Franchis R, Mandato C, Buoninconti A. Risk factors for neural tube defects: analysis of common variants of methylenetetrahydrofolate reductase and cystathionine beta-synthase. (Abstract). Am J Hum Genet 1997:A151.
106. Morrison K, Papapetrou C, Hol FA, et al. Susceptibility to spina bifida: an association study of five candidate genes. Ann Hum Genet 1998;62:379–96.
107. Shields DC, Kirke PN, Mills JL, et al. The "thermolabile" variant of methylenetetrahydrofolate reductase and neural tube defects: an evaluation of genetic risk and the relative importance of the genotypes of the embryo and the mother. Am J Hum Genet 1999;64:1045–55.
108. van der Put NM, Eskes TK, Blom HJ. Is the common 677C→T mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. QJM 1997;90:111–15.
109. Speer M, Worley G, Mackey J, et al. The thermolabile variant of methylenetetrahydrofolate reductase (MTHFR) is not a major risk factor for neural tube defect in American Caucasians. Neurogenetics 1997:149–50.